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Human ficolin-1 interacts with Ebola virus glycoprotein: A novel case of lectin-dependent enhancement of viral infection

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Ebola virus (EBOV) infection is initiated by attachment of the surface viral glycoprotein (GP) to target cells. The trimeric GP is a highly glycosylated protein, known to interact with host C-type lectin receptors and the soluble complement protein mannose-binding lectin (MBL), thereby enhancing viral infection. Similarly to MBL, ficolins are part of the defense collagens family of proteins that recognize particular glycans at the pathogen surface, trigger activation of the lectin complement pathway and mediate phagocytosis of the recognized targets. Our objective was to investigate the interaction of EBOV GP with the three human ficolins.

Ficolin-1 only was able to bind to EBOV GP, with an affinity comparable to that of MBL, as shown by surface plasmon resonance analysis of the interactions with the immobilized recombinant ectodomain of GP. Binding was calcium-dependent, inhibited by acetylated glycans and mediated by the fibrinogen-like recognition domain of ficolin-1. Using the ficolin-1 Y271F mutant devoid of sialic acid binding capacity and a recombinant GP devoid of mucin domain, we identified sialylated moieties of the mucin domain as ficolin-1 ligands. In contrast, the GP mucin domain was dispensable for MBL binding. Ficolin-1 was shown to enhance EBOV infection of Vero cells using pseudotyped viruses and the requirement for the GP mucin domain was confirmed using viral particles with surface exposed GP lacking this domain. Moreover the ficolin-1-dependent enhancement of infection was observed using viral particles expressing the Zaïre or Reston EBOV strain GP on their surfaces. Ficolin-1 was also shown to enhance infection of Vero cells when genuine EBOV was used. In addition, similar results were obtained with human monocyte-derived-macrophages, which are major viral target cells. Competition experiments suggested that, although ficolin-1 and MBL recognized different carbohydrate moieties on EBOV GP, the observed enhancement of infection likely depended on a common cellular receptor/partner.

In conclusion, we have identified ficolin-1 as a novel actor in EBOV host cell attachment that serves as a bridging molecule between the mucin domain of GP and a host target cell receptor that remains to be identified. The facilitation of EBOV entry into target host cells by interaction with ficolin-1 and other host lectins likely facilitates its survival in infected host cells and contributes to the virus strategy to subvert the innate immune response.

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Molecular dissection of the interaction of C1q with CD91

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Removal of apoptotic cells, or efferocytosis, plays a crucial role in essential physiological processes including maintenance of tissue homeostasis and immune tolerance. The low density lipoprotein receptor-related protein 1 (LRP1 or CD91), a membrane receptor implicated in efferocytosis, was previously reported to interact with both C1q and MBL complement defense collagens. The aim of the present study is to develop tools in order to decipher the interaction of LRP1 with C1q at the molecular and cellular levels.

LRP1 is a large endocytic modular receptor composed of 4 extracellular clusters of ligand binding cysteine rich complement-type repeats followed by a transmembrane domain and a cytoplasmic tail. The four clusters of LRP1 with a His-tag at their C-terminus were cloned in the pcDNA3.1 vector for stable expression as soluble fragments in HEK 293-F cells. Clusters II, III and IV were successfully produced and purified by affinity chromatography. Recombinant RAP (receptor-associated protein), described as a general antagonist of LRP1 ligand binding, was also produced using a bacterial expression system. Analysis of the interaction of the soluble clusters with immobilized C1q by surface plasmon resonance shows that the interaction of C1q with LRP1 involves clusters II and IV and that these interactions are inhibited by RAP. Cellular models are also under development for interaction studies at the cell surface, and phagocytosis experiments. A mini-receptor corresponding to the sole cluster IV associated with the transmembrane domain and cytoplasmic tail of LRP1 cloned in pcDNA3.1 as well as a full length LRP1 construct were used for stable transfection in mammalian cells. The expression of full length LRP1 or LRP1 mini-receptor IV at the surface of LRP1-null CHO cells was characterized by immunofluorescence and flow cytometry. Ectopic expression of LRP1 and LRP1 minireceptor IV by CHO cells was further shown to significantly increase the interaction with late apoptotic Jurkat cells. Overall, these molecular and cellular tools will allow detailed investigation of the role of the defense collagens in LRP1-mediated efferocytosis.

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Parasite calreticulins: Structure, C1q binding and dual carbohydrate/peptide interaction properties

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Calreticulin (CRT) is a multifaceted protein of 45k Da, early discovered as a receptor of the collagen-like regions of the complement C1q protein and better known as an ER chaperone protein,

essential in calcium metabolism. Various implications in cancer, early development and immunology were discovered more recently for CRT, as well as its role as a dominant "eat-me" prophagocytic signal. Intriguingly, cell-surface exposure/secretion of CRT is among the infective strategies used by several human parasites. We focused on the CRTs from *Trypanosoma cruzi* (TcCRT) and *Entamoeba histolytica* (EhCRT).

Analysis of the interaction between C1q and both parasite CRTs provided several lines of evidence for the main contribution of the C1q and CRT globular regions, at least *in vitro*. Because of the inherent flexibility of CRTs, their analysis by X-ray crystallography requires the design of recombinant constructs suitable for crystallization. Based on the strategy used for human and mouse CRTs, we have solved the X-ray structures of TcCRT and EhCRT. Although the hypothesis that CRT can exhibit both open and closed conformations has been proposed in relation to its chaperone function, only the open conformation has yet been observed in crystal structures. We provide the first evidence of a closed conformer in the case of EhCRT, which involves a complex conformational transition. SAXS experiments also provided additional information about the flexibility of TcCRT in solution, thus complementing crystallographic data on the open conformation. Finally, regarding the conserved lectin domain structure and chaperone function, we now provide direct evidence of its unusual hybrid recognition properties, with several examples of dual carbohydrate and peptide binding.

Comparison of the structures of phylogenetically distant parasite CRTs with their mammalian counterparts highlights key features involved in their common structure and chaperone function. In addition, these structures reveal species specific structural features that might be harnessed to fight against the parasites without affecting the functions of the host CRT.

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Three cases of C3 glomerulonephritis associated with group A streptococcus infection

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Background: C3 glomerulonephritis (C3GN) is a recently introduced clinical entity based on the dysregulation of the complement alternative pathway, but its etiology is unclear. Here, we report three children with C3G in which group A streptococcus (GAS) infection could be involved in the pathogenesis.

Cases: We encountered three children (10-year-old male, 11-year-old female, and 12-year-old male) with C3GN. Urinalysis showed both proteinuria and hematuria in all, but none had nephrotic syndrome. One patient showed transient macro hematuria. The renal function of all three patients was normal, but serum C3 levels were below normal range (26 mg/dl, 17 mg/dl, and 21 mg/dl, respectively) despite normal serum C4 levels. Interestingly, serum titer of anti-streptolysin O antibody (ASO) was elevated (515–527; normal range <240) and GAS infection in all patients was confirmed by pharyngeal cultures. Their renal pathological findings were consistent with typical C3GN; diffuse mesangial proliferation, duplication of capillary walls, focal or diffuse endocapillary proliferations and isolated immunofluorescent (IF) staining with C3. Additionally, immunofluorescence (IF) staining of nephritis-associated plasmin receptor (NAPlr) antigen, identified as a nephritic antigen causing poststreptococcal acute glomerulonephritis, was positive in the glomeruli of all patients. One patient was treated with two courses of intravenous methylprednisolone pulse therapy (MPT) followed by oral prednisolone combined with mizoribine, losartan, and dipyridamole. The other two were administered lisinopril. GAS was successfully eradicated with antibiotics, but laboratory and urinary findings were not improved with antibiotics only. At the last visit, the patient treated with MPT and combined therapy showed normal urinalysis and improvement of hypocomplementemia (C3, 52 mg/dl). The other two showed moderate reduction of proteinuria, but hypocomplementemia persisted.

Discussion: To date, there are a few anecdotal reports of C3GN concomitant with GAS infection, and the outcome of such cases is not well described. In our patients, not only positive pharyngeal culture and elevated ASO were observed, but also positive NAPlr staining in the glomeruli. Positive NAPlr staining in the glomeruli strongly supports the hypothesis that there may be patients who developed C3GN in association with GAS infection.

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Bactericidal assay with autologous serum demonstrates increased bactericidal activity after Hib vaccination in C2 deficiency

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Deficiencies of the components of the classical pathway of complement are associated with increased risk of infections with encapsulated bacteria, such as *Haemophilus (H.) influenzae*. We have previously demonstrated adequate antibody response to *H. influenzae* type b (Hib) vaccination in C2 deficiency (C2D). Defense against Hib is dependent on serum bactericidal activity, which involves antibodies and complement. Due to absence of normal classical pathway function in C2D, any serum bactericidal activity would have to depend on alternative pathway function or C2 bypass mechanisms, such as C1q-dependent activation of the alternative pathway. Bactericidal capacity of antibodies in autologous complement in relation to vaccination has not been investigated at group level in C2D.

Serum from 18 persons with C2D and 26 healthy controls, collected before and 4 to 6 weeks after vaccination with Act-HIB®, was kept at −80 °C. Serum bactericidal activity (SBA) against Hib bacteria was analysed in buffer supporting both the classical and the alternative pathways, using autologous serum as complement source. IgG antibodies to Hib capsular polysaccharide,